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Application Note 00569

Analysis of Gabapentin Extracted from Postmortem Specimens on the Varian 1200L Triple Quadrupole LC/MS/MS

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Introduction

Gabapentin (Figure 1) , an analog of the neurotransmitter GABA, is used as an anticonvulsant in the treatment of epilepsy. It was approved by the FDA as an anti-seizure medication but has many controversial off-label uses, such as in the treatment of bipolar disorder, social anxiety disorder, obsessive-compulsive disorder and insomnia. Gabapentin is also widely used as a pain reliever and was one of the 50 most prescribed drugs in the United States in 2003 due to its mild side-effect profile. Gabapentin may increase the effect of other drugs such as antidepressants, alcohol and pain relievers.

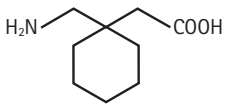

Figure 1 Chemical structure of Gabapentin, (1-(aminomethyl)-cyclohexaneacetic acid).

LC/MS/MS is utilized in the analysis of gabapentin because it allows for a simple, cost-effective clean up, requires no derivatization of the drug and provides accurate results with excellent sensitivity. This application demonstrates that the Varian 1200L triple quadrupole LC/MS/MS can identify and quantify gabapentin in postmortem specimens with the use of two MRM transitions. The Los Angeles County Department of Coroner Toxicology Laboratory provided the data presented.

Instrumentation

- Varian 1200L LC/MS equipped with an ESI source
- Varian ProStar 410 Autosampler
- Varian ProStar 210 Binary Gradient Pumps

Materials and Reagents

A 1.0 mg/mL working solution of gabapentin (supplied by Parke-Davis Pharmaceutical Company) was prepared in methanol (HPLC grade, Fisher Scientific Company). The internal standard, Baclofen (Sigma-Aldrich Chemical Company), was dissolved in water (HPLC grade, Fisher Scientific) to a working concentration of 1.0 mg/mL. All other solvents used in this procedure were of HPLC grade and purchased from Fisher Scientific.

Sample Preparation

The extraction of gabapentin from biological specimens was performed using the principles of protein precipitation by acetonitrile (without a clean up step) using the following procedure.

1. Accurately measure 1.0 ml of standard, quality control and case samples to appropriately labeled 13 x 100 mm test tubes.
2. Add 10 µl of the internal standard Baclofen (final concentration = 10 µg/ml) to each tube and vortex.
3. Aliquot 50 µl of each standard, quality control and case sample into new set of 13 x 100 mm test tubes.
4. Add 400 µl of acetonitrile to each tube and vortex.
5. Centrifuge for 10 minutes.
6. Decant or pipet the supernatant to a clean 13 x 100 mm test tube.
7. Evaporate the supernatant to dryness.
8. Reconstitute the samples with 200 µl of Methanol: Water (60:40). Vortex.
9. Transfer to labeled autosampler vials with inserts.

Conditions

MS Conditions:

Ionisation Mode: ESI positive
Scan Time: 1.0 s
Drying Gas: 350 °C at 25 psi
Collision Cell Pressure: 2.0 mTorr

Compounds	Capillary Voltage (V)	Precursor Ion (m/z)	Product Ions (m/z)	Collision Energy (V)
Gabapentin	65	**172**	**154**	-26.50
Gabapentin	65	172	137	-29.00
Baclofen (IS)	65	**214**	**151**	-36.50

Table 1 MRM conditions for the LC/MS/MS analysis of gabapentin and baclofen (IS). Quantification ions are displayed in bold.

HPLC Conditions:

Column: Pursuit Diphenyl 50 mm x 2.0 mm 3µ (Varian Part Number: A3041050X020)
Guard Column: Pursuit Diphenyl 2.0 mm I.D., 3µ (Varian Part Number: A3041MG2)
Solvent A: Water
Solvent B: Methanol
Flow Rate: 200 µL/min
Injection Volume: 25 µL

LC Program:Time (min)	%A	%B
0:00	60	40
1:00	60	40
1:30	5	95
3:30	5	95
3:35	60	40
5:00	60	40

Discussion

The calibration curve in Figure 2 was obtained from spiked porcine blood samples that had been protein precipitated with acetonitrile. Six concentrations, representative of the standard therapeutic range of gabapentin, were selected: 1.0, 5.0, 10, 15, 20, and 25 µg/mL. The calibration curve used a linear curve fit without regression weighting and forced the curve through the origin. The RSD value from the curve was 6.929% with and r^2 of 0.999.

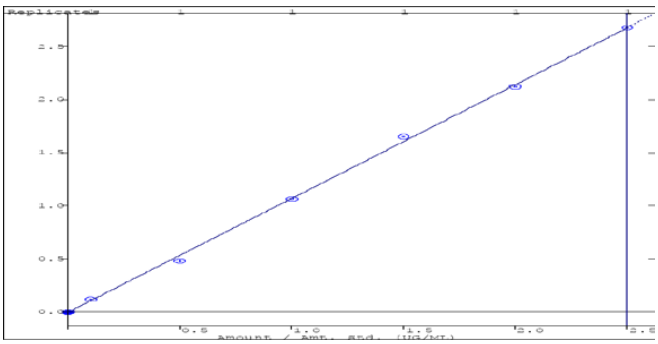

Figure 2 Calibration curve of gabapentin in porcine blood using baclofen as the internal standard. RSD = 6.929%; r^2 = 0.999

The peak-to-peak signal-to-noise ratio of gabapentin at 1.0 µg/mL, the reporting limit, for the quantitation and confirmatory ions are 543 and 362, respectively as shown in Figure 3. The analysis provides excellent sensitivity and reproducibility, with a simple, fast, and inexpensive sample clean-up procedure. This demonstrates that the instrument is capable of detecting even lower levels with the extraction procedure.

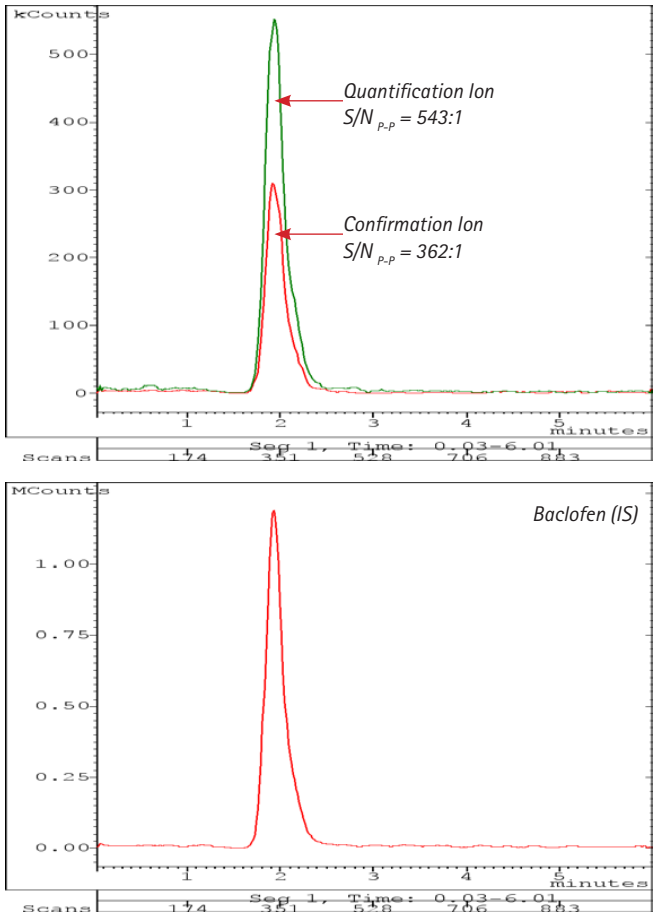

Figure 3 MRM chromatograms showing the quantification (green) and confirmation (red) ions of gabapentin at a concentration of 1.0 µg/mL (top) and the baclofen internal standard at 10.0 µg/mL (bottom).

Conclusion

The data demonstrates the ability of the 1200L triple quadrupole LC/MS/MS to effectively identify and quantitate gabapentin at the therapeutic levels using a protein precipitation method of extraction from a postmortem matrix without any evidence of matrix effects. Varian Inc. offers a complete solution for the qualification and quantification of gabapentin.

Varian Inc. would like to provide a special thank you to Sara Kegler and Dan Anderson from Los Angeles County Department of Coroner for the validation of both the extraction procedure and the instrument parameters, and for providing the data displayed in this application note.

These data represent typical results.
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